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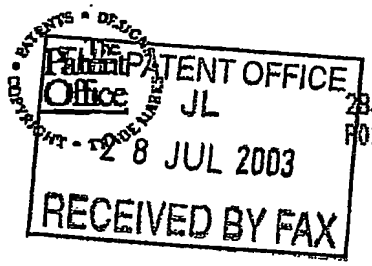

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3. Full name, address and postcode of the or of each applicant *(underline all surnames)*:
University of Southampton
Highfield
Southampton
So17 1BJ

Patents ADP number *(if you know it)*: 79847000 1

If the applicant is a corporate body, give the country/state of its incorporation: UK

4. Title of the invention: Extending the Memory of Nuclear Spins: Singlet States in Low Magnetic Field

5. Name of your agent *(if you have one)*:
Centre for Enterprise and Innovation
(CEI)
University of Southampton
Highfield
Southampton
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Extending the Memory of Nuclear Spins: Singlet States in Low Magnetic Field

One-Sentence Summary: Low-field nuclear magnetic singlet states allow the storage of nuclear spin order for much longer than the conventional relaxation time limit.

Abstract

Nuclear spin singlet states may be used to store nuclear spin order in a room temperature liquid for a time much longer than the spin-lattice relaxation time constant T_1. The nuclear spin singlets are unaffected by intramolecular dipole-dipole relaxation, which is generally the predominant relaxation mechanism. We demonstrate storage of nuclear spin order for more than ten times longer than the measured value of T_1. This phenonemon may facilitate the development of nuclear spin hyperpolarization methods, and may allow the study of motional processes which occur too slowly for existing NMR techniques.

One of the principal factors contributing to the wide-ranging utility of nuclear magnetic resonance (NMR) is the relatively long lifetime of nuclear spin order. This long lifetime allows NMR spectroscopists to follow processes such as diffusion, flow and slow molecular motion *(1,2)*, and to contemplate using NMR for quantum computation *(3,4)*. The relaxation of the nuclear spins back to thermal equilibrium, and hence the lifetime of the nuclear spin memory, is characterized by a time constant T_1 known as the longitudinal relaxation time constant, or as the spin-lattice relaxation time constant. In most cases, this parameter is of the order of seconds, although it may be much longer in special cases. Major changes in T_1 can usually only be achieved by changing the isotopic composition of the sample, or by changing its physical state. The sophisticated manipulation of nuclear spin interactions by radio-frequency pulse sequences, so prevalent in modern NMR *(1)*, have always been restricted to a time-frame set by the intrinsic T_1 limit. Experiments which seek to enhance NMR signals by hyperpolarization phenomena *(5-9)* have great potential for extending the range of NMR even further, but are always hampered by the need to use the hyperpolarized substance within a time of the order of T_1.

In this report, we demonstrate, for the first time, the storage of nuclear spin order for a time much longer than T_1. So far, we have demonstrated

this phenomenon on molecules containing pairs of protons, in which the main mechanism for nuclear spin relaxation is the modulation of the intramolecular dipole-dipole coupling by molecular motion. The extension of nuclear spin memory beyond the T_1 limit is achieved by creating nuclear spin singlet states in low magnetic field (20 mT or less). As shown below, these low-field singlet states (LFS) are immune to the dominant intramolecular dipole-dipole mechanism and hence relax with a time constant much longer than the conventional T_1. The LFS states are generated by adiabatic transfer from non-equilibrium high-field states. The singlet states are allowed to decay in low field, and then transferred adiabatically back to high field where they can be detected as perturbations of conventional NMR signals. Low-field singlet states have been observed to decay with a time constant $T_{LFS} = 104 \pm 5$ s, in a sample for which the measured T_1 is 16.8 ± 0.3 s under identical conditions.

The experimental procedure for investigating the lifetime of low-field singlet states is sketched in Fig.1. In its simplest form, the experiment applies to an ensemble of molecules, each containing a pair of spins-1/2 of the same isotopic type, but with different chemical shifts δ_1 and δ_2, and a mutual J-coupling J. The experimental procedure uses a physical transport of the sample between two different magnetic field strengths, B_{high} and B_{low}, where $B_{high} >> B_{low}$, as shown in Fig.1a. The transport processes from high to low field, and *vice versa*, take times $\tau^{(1)}_{transp}$ and $\tau^{(2)}_{transp}$ respectively, which are assumed to be less than T_1 (both intervals are of the order of 10 seconds

Fig.1

in the experiments reported here). The low-field storage interval τ_{LF}, on the other hand, may be much longer than T_1. The transport between the two magnetic fields is synchronized with two radiofrequency pulse sequences denoted A_+ and B in Fig.1b. These pulse sequences are applied at the Larmor frequency of the spins in the high magnetic field, and are specified in *Supporting Material*.

The energy level picture at time point ① in Fig.1c shows the four Zeeman energy levels, with energies in the order $E_{\alpha\alpha} < E_{\alpha\beta} < E_{\beta\alpha} < E_{\beta\beta}$, assuming that $\delta_2 > \delta_1$, the gyromagnetic ratio γ and the J-coupling are both positive, and that the spin system is weakly-coupled in high field. In thermal equilibrium at ambient temperature, the lowermost level $|\alpha\alpha>$ has a small excess population (depicted by the filled balls), while the uppermost level $|\beta\beta>$ has a slightly depleted population (depicted by the white balls). These population differences are very small in the case of conventional thermal polarization, but may be of the order of one in spin hyperpolarization experiments *(5-9)*.

As discussed in *Supporting Material*, the pulse sequence A_+, which is given by two 90° pulses separated by a delay, exchanges the population of state $|\alpha\alpha>$ with that of $|\alpha\beta>$, and that of $|\beta\alpha>$ with that of $|\beta\beta>$. Hence, at time point 2, a non-equilbrium population distribution is generated, in which level $|\alpha\beta>$ has an excess population, while $|\beta\alpha>$ has a depleted population, as shown for time point ② in Fig.1c.

During the interval $\tau^{(1)}_{\text{transp}}$, the sample is transported to a region of low magnetic field B_{low}, which is of the order of milliTesla. In low field, it is convenient to discuss the behaviour of the nuclear spins using the four eigenstates of the pure J-coupling Hamiltonian:

$$\mathcal{H}_J = 2\pi J \boldsymbol{I}_1 \cdot \boldsymbol{I}_2 \qquad [1]$$

The four J-coupling eigenstates may be classified as the three components of a triplet state, plus a singlet state. The triplet state components are denoted

$$\begin{aligned} |T_1\rangle &= |\alpha\alpha\rangle \\ |T_0\rangle &= \frac{1}{\sqrt{2}}(|\alpha\beta\rangle + |\beta\alpha\rangle) \\ |T_{-1}\rangle &= |\beta\beta\rangle \end{aligned} \qquad [2]$$

and have the same J-coupling eigenvalue of $+\frac{1}{2}\pi J$. The singlet state is denoted

$$|S_0\rangle = \frac{1}{\sqrt{2}}(|\alpha\beta\rangle - |\beta\alpha\rangle) \qquad [3]$$

and has a J-coupling eigenvalue of $-\frac{3}{2}\pi J$. The triplet and singlet states are energy eigenstates of the nuclear spins only if the magnetic field is zero. The energy level diagram in this case is depicted next to time point ③ in Fig.1c. Although we use the zero-field eigenstates as a basis for a representation of

the spin density operator, the treatment below does not assume a field of exactly zero.

If the transport from high to low field is fast compared to T_1, but slow compared to the J-coupling, the excess population of the high-field eigenstate $|\alpha\beta>$ is transferred adiabatically into an excess population of the low-field eigenstate $|S_0>$. Similarly, the depleted population of the high-field eigenstate $|\beta\alpha>$ is transferred adiabatically into a depleted population of the zero-field eigenstate $|T_0>$. These adiabatic transfer processes have been exploited before in the context of parahydrogen-enhanced NMR *(7)*. The idealized distribution of the singlet and triplet state populations is shown at time point ③ in Fig.1c. This assumes pure adiabatic transfer and neglects T_1 relaxation during the transport interval.

The nuclear spins evolve in low magnetic field under a combination of coherent effects and incoherent relaxation processes. The coherent part of the low-field evolution has been studied before *(10,11)* but the analysis of incoherent molecular motion in very low field NMR has been restricted to the motional averaging of interaction tensors and to the investigation of lineshape perturbations *(12-15)*. Here we use a standard second-order perturbation treatment of the modulated dipole-dipole interaction *(16)* to analyze the spin-lattice relaxation in low field.

In general, the spin density operator ρ evolves according to the Liouville-von Neumann equation *(1)*

$$\frac{\mathrm{d}}{\mathrm{d}t}\rho(t) = \hat{\tilde{L}}_{\mathrm{LF}}\rho(t) \qquad [4]$$

where the low-field Liouvillian is given by

$$\hat{\tilde{L}}_{\mathrm{LF}} = \hat{\tilde{\Gamma}}_{\mathrm{LF}} - \mathrm{i}\tilde{\mathcal{H}}_{\mathrm{LF}} \qquad [5]$$

and $\tilde{\mathcal{H}}_{\mathrm{LF}}$ is the superoperator for the commutator with the low-field spin Hamiltonian:

$$\mathcal{H}_{\mathrm{LF}} = -\gamma B_{\mathrm{low}}(1+\delta_1)I_{1z} - \gamma B_{\mathrm{low}}(1+\delta_2)I_{2z} + \mathcal{H}_J \qquad [6]$$

The thermal polarization terms *(17)* may be ignored in low field. If the relaxation is dominated by the intramolecular dipole-dipole coupling mechanism, the relaxation superoperator may be evaluated by second-order time-dependent perturbation theory and is given by *(1)*

$$\hat{\tilde{\Gamma}}_{\mathrm{LF}} \cong -\int_{-\infty}^{0} \overline{\hat{\tilde{\mathcal{H}}}_{\mathrm{DD}}(-\tau)\hat{\tilde{\mathcal{H}}}_{\mathrm{DD}}(0)}\,\mathrm{d}\tau \qquad [7]$$

where $\hat{\tilde{\mathcal{H}}}_{\mathrm{DD}}(t)$ is the commutation superoperator for the dipole-dipole coupling Hamiltonian, evaluated at time t, and the overbar represents an ensemble average. Eq.[4] is general for any field strength low enough to satisfy the extreme narrowing condition for the dipole-dipole relaxation.

It is convenient to use the matrix representation of the Liouvillian superoperator $\bar{\bar{L}}_{LF}$ in a space of orthonormal spin operators *(18)*. The following set of six operators generate a suitable basis:

$$
\begin{aligned}
|1) &= |S_0><S_0| &&= \tfrac{1}{2}(I_1^\alpha I_2^\beta + I_1^\beta I_2^\alpha - I_1^+ I_2^- - I_1^- I_2^+) \\
|2) &= |T_1><T_1| &&= I_1^\alpha I_2^\alpha \\
|3) &= |T_0><T_0| &&= \tfrac{1}{2}(I_1^\alpha I_2^\beta + I_1^\beta I_2^\alpha + I_1^+ I_2^- + I_1^- I_2^+) \\
|4) &= |T_{-1}><T_{-1}| &&= I_1^\beta I_2^\beta \\
|5) &= \tfrac{1}{2}(|S_0><T_0| + |T_0><S_0|) &&= \tfrac{1}{\sqrt{2}}(I_1^\alpha I_2^\beta - I_1^\beta I_2^\alpha) \\
|6) &= \tfrac{1}{2i}(|S_0><T_0| - |T_0><S_0|) &&= -i\tfrac{1}{\sqrt{2}}(I_1^+ I_2^- - I_1^- I_2^+)
\end{aligned}
$$

where $I_j^\pm$ are single-spin shift operators and $\{I_j^\alpha, I_j^\beta\}$ are single-spin polarization operators *(1,2)*. The ket $|1)$ describes the population operators of the singlet state. Kets $|2)$, $|3)$ and $|4)$ describe the population operators of the three triplet states. Kets $|5)$ and $|6)$ represent the x and y-components of the coherence between the singlet state and the central component of the triplet. All of these operators commute with the angular momentum operator along the field axis, as does the low-field Hamiltonian $\mathcal{H}_{LF}$. The operator basis in Eq.[8] is closed under the application of the low-field Liouvillian $\bar{\bar{L}}_{LF}$.

The matrix representation of the low-field relaxation superoperator $\bar{\bar{\Gamma}}_{LF}$ may be evaluated by standard relaxation theory *(1)*, assuming pure intramolecular dipole-dipole relaxation driven by rigid isotropic random rotation with

a correlation time τ_c. The low-field Liouvillian evaluates to

$$\hat{\hat{L}}_{LF} = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & -2^{-1/2}\omega_\Delta^{low} \\ 0 & -\frac{9}{10}b^2\tau_c & \frac{3}{10}b^2\tau_c & \frac{3}{5}b^2\tau_c & 0 & 0 \\ 0 & \frac{3}{10}b^2\tau_c & -\frac{3}{5}b^2\tau_c & \frac{3}{10}b^2\tau_c & 0 & 2^{-1/2}\omega_\Delta^{low} \\ 0 & \frac{3}{5}b^2\tau_c & \frac{3}{10}b^2\tau_c & -\frac{9}{10}b^2\tau_c & 0 & 0 \\ 0 & 0 & 0 & 0 & -\frac{1}{2}b^2\tau_c & 2\pi J \\ 2^{-1/2}\omega_\Delta^{low} & 0 & -2^{-1/2}\omega_\Delta^{low} & 0 & -2\pi J & -\frac{1}{2}b^2\tau_c \end{pmatrix} \quad [8]$$

where the dipole-dipole coupling between the spins is given by $b = -(\mu_0/4\pi)\gamma^2\hbar r^{-3}$, and r is the internuclear distance. The low-field chemical shift frequency difference is defined

$$\omega_\Delta^{low} = -\gamma B_{low}(\delta_2 - \delta_1) \quad [9]$$

In zero field ($\omega_\Delta^{low} = 0$), the first row and column of Eq.[8] contain only zeros. This indicates that the singlet state population is dynamically isolated in low field, and is conserved under the evolution process. The zero-field singlet state may be used as a long-term repository for nuclear spin order, protected from intramolecular dipole-dipole relaxation.

If the magnetic field is not exactly zero, Eq.[8] indicates that the singlet state is relaxed indirectly by dynamic couplings to the central triplet state and to singlet-triplet coherence.

The idealized behaviour of the spin ensemble after an interval τ_{LF} in low field is depicted by the diagram at time point ④ in Fig.1c. If the field is sufficiently low, and τ_{LF} is long enough, the triplet populations equilibrate, while the singlet population is conserved.

The singlet population may be read out by adiabatic transport of the sample back into high field during the interval $\tau^{(2)}_{transp}$. This transforms the low-field singlet population into a population of the high-field $|\alpha\beta>$ state, as depicted for time point ⑤ in Fig.1c. The population of the $|\alpha\beta>$ state is converted into observable NMR signals by the pulse sequence B. As described in *Supporting Material*, this sequence, which is given by three rf pulses separated by two unequal delays, converts the $|\alpha\beta>$ state population into antiphase NMR signals centred at the chemical shift δ_2. NMR signals deriving from T_1 relaxation during the transport interval, on the other hand, only generate NMR signals at the chemical shift δ_1. The interesting NMR signals deriving from low-field singlet storage are therefore cleanly separated from signals with a trivial origin.

Experimental results for a 17.7 mM solution of 2,3-dibromothiophene in DMSO-d^6 at 20°C are shown in Fig.2. The proton spin system is defined by $\delta_1 = 7.11$ ppm, $\delta_2 = 7.72$ ppm and $J = 5.7$ Hz. A conventional proton NMR spectrum, obtained at 400 MHz in the field $B_{high} \cong 9.4$ T, is shown in Fig.2a and displays the two doublets of a typical AX spin system. A conventional inversion-recovery series (not shown), shows that the spin-lattice

Fig.2

relaxation time constants for the inequivalent sites are very similar in high field ($T_1(\delta_1) = 16.5 \pm 0.2\,\mathrm{s}$; $T_1(\delta_2) = 17.1 \pm 0.2\,\mathrm{s}$).

Fig.2b shows the spectrum obtained after the singlet-storage sequence in Fig.1, using a storage time $\tau_{LF} = 100\,\mathrm{s}$, and a storage field $B_{low} = 1.8 \pm 0.5\,\mathrm{mT}$. The spectrum in Fig.2b shows diagnostic antiphase signals at around $\delta_2 = 7.72\,\mathrm{ppm}$, and an antiphase dispersion structure around $\delta_1 = 7.11\,\mathrm{ppm}$.

We can prove the phenomenon of low-field singlet storage by replacing the sequence A_+ by the sequence A_-. As discussed in *Supporting Material*, the sequence A_-, which is derived from A_+ by changing one of the pulse phases, exchanges the population of state $|\alpha\beta>$ with that of $|\beta\beta>$, instead of with $|\alpha\alpha>$. The population of $|\alpha\beta>$ is therefore depleted at time point② rather than enhanced. Fig.2c shows the expected change in sign of the diagnostic antiphase signals around $\delta_2 = 7.72\,\mathrm{ppm}$. The signals around $\delta_1 = 7.11\,\mathrm{ppm}$ are also perturbed, but in a more complicated way, since these signals are superpositions of several contributions (see *Supporting Material*). Since the total interval $\tau^{(1)}_{transp} + \tau_{LF} + \tau^{(2)}_{transp} = 120\,\mathrm{s}$ exceeds T_1 by a factor of seven, the spectra in Fig.2b and c prove that the memory of the nuclear spin system has been extended significantly beyond the T_1 limit. Fig.3

Fig.3 shows the measured decay of the singlet population in low field, derived by monitoring the 7.72 ppm antiphase signals as a function of τ_{LF}. In a field $B_{low} = 1.8 \pm 0.5\,\mathrm{mT}$, the time constant for the singlet decay is estimated to be $T_{LFS} = 104 \pm 5\,\mathrm{s}$. The singlet decay time constant had

no appreciable field-dependence for magnetic fields less than $B_{low} = 20\,\mathrm{mT}$. This behaviour is expected from numerical calculations based on Eq.[8] (see *Supporting Material*).

The singlet decay is observed to accelerate when the proton concentration in the solution is increased. This indicates that intermolecular proton-proton relaxation is a strong contributor to the singlet relaxation, and that very long lifetimes may only be achievable in highly dilute solutions. Singlet relaxation is also expected to be caused by paramagnetic impurities such as dissolved oxygen, intermolecular interactions with solvent deuterons, and scalar relaxation of the second kind *(16)* via the Br nuclei.

The long-term persistence of the zero-field singlet state cannot be attributed to the field-dependence of T_1, which is very mild for the sample studied here. A more correct interpretation is that the low external field suppresses the chemical shift difference and renders the spins magnetically-equivalent, which isolates the singlet state from the triplet manifold, even with respect to dipole-dipole relaxation. In effect, the singlet nuclear spin state of 2,3-dibromothiophene is an analogue of parahydrogen, which is also a nuclear spin singlet *(6-8)*.

The storage effect should be observable in systems of larger numbers of spins as long as magnetic equivalence can be switched on and off by a change in the external magnetic field strength. We are currently exploring four-proton systems of the form X-CH_2-CH_2-Y which should display a similar property.

Singlet storage effects should be observable entirely in high magnetic field if a suitable radiofrequency pulse sequence is used to suppress chemical shift differences *(19)*. Analogous "zero-field experiments in high field" have been performed in solid-state NMR *(20,21)*. Radiofrequency pulse sequences might be designed which allow the storage of nuclear spin order for times much longer than T_1, without removing the sample from the high-field magnet.

Extension of the spin memory time should be useful whenever nuclear spin states are used as a long-term label for the position or chemical state of molecules. The long lifetime of low-field singlet states is expected to facilitate nuclear hyperpolarization experiments, especially those involving parahydrogen *(6-8)*, and may create new possibilities in the exploitation of NMR for quantum computation *(3,4)*.

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Figure Captions.

Fig.1. (a) Sequence of magnetic fields used in the experiment. The sample is transported from a region of high magnetic field $B_{\rm high}$ into a region of low magnetic field $B_{\rm low}$ in a time $\tau^{(1)}_{\rm transp}$. After remaining in the low field for a time τ_{LF}, the sample is transported back into the high-field region, a process which takes a time $\tau^{(2)}_{\rm transp}$.

(b) Radio-frequency pulse sequence performed at the Larmor frequency of the spins in high field. The pulse sequences A_+ and B are specified in *Supplementary Material.*

(c) Idealized sequence of spin state populations during the experiment. The four high-field energy levels belong to the states $|\beta\beta>$, $|\alpha\beta>$, $|\alpha\alpha>$ and $|\beta\alpha>$, reading in a clockwise direction starting with the upper state.

Fig.2. (a) Conventional ^{1}H NMR spectrum of the solution of 2,3-dibromothiophene (inset) in DMSO-d^6.

(b) Spectrum generated by the sequence in Fig.1, using the preparation sequence A_+ and a storage time $\tau_{LF} = 100\,s$.

(c) Spectrum generated by the sequence in Fig.1, using the preparation sequence A_- and a storage time $\tau_{LF} = 100\,s$.

Expanded sections of the spectra from 7.76 to 7.68 ppm are shown for (b) and (c). All spectra were obtained from a single signal acquisition. The spectra in (b) and (c) are expanded vertically by a factor of 2 compared to (a).

Fig.3. Decay of the diagnostic antiphase signals as a function of time τ_{LF}. The top and lower half indicate the integrated amplitudes of the two components of the antiphase multiplet centred at 7.72 ppm. The symbols represent measurements at different storage fields B_{low}. The vertical scale represents the integrated amplitude of the spectral peaks as a percentage of their amplitude in an ordinary one-pulse NMR experiment. The solid lines are fits to decaying exponential functions with time constant $T_{LFS} = 104\,s$. The singlet decay is independent of the storage field in this regime.

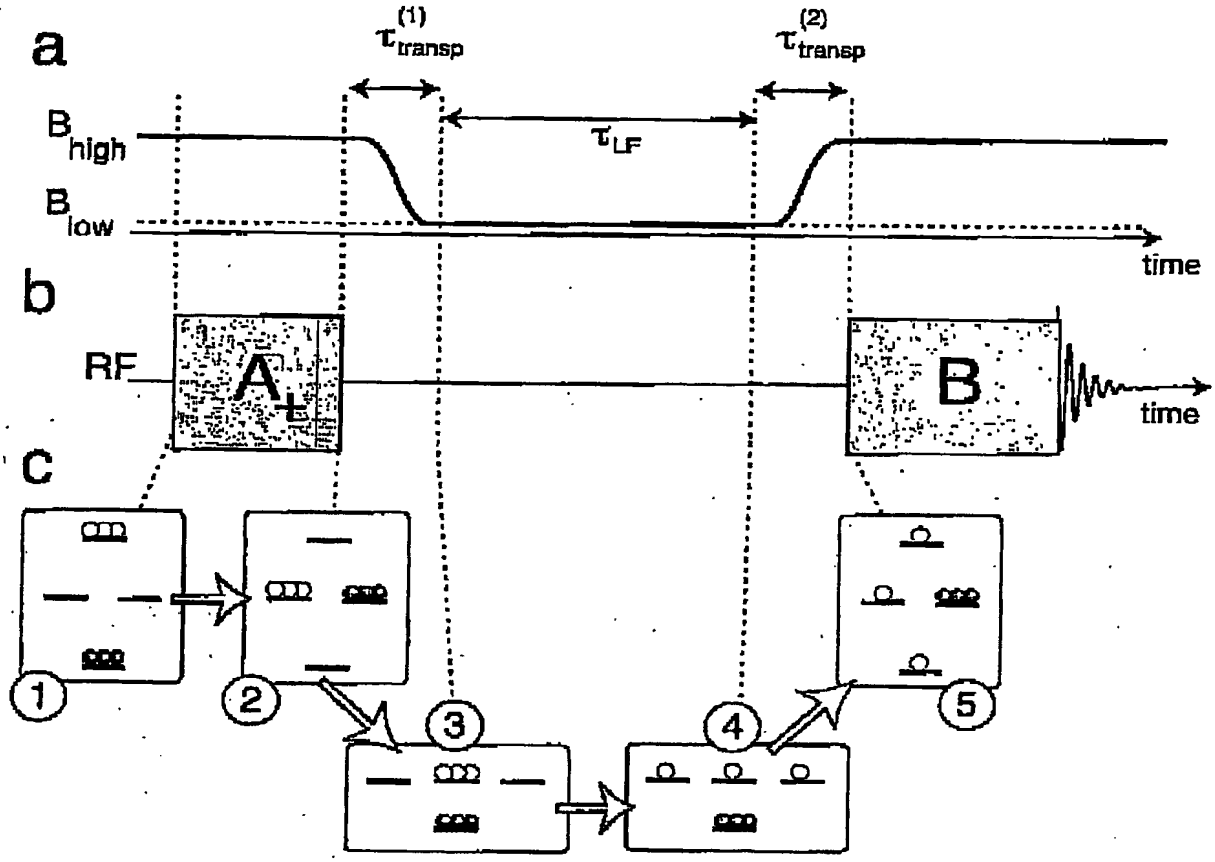


Fig.1

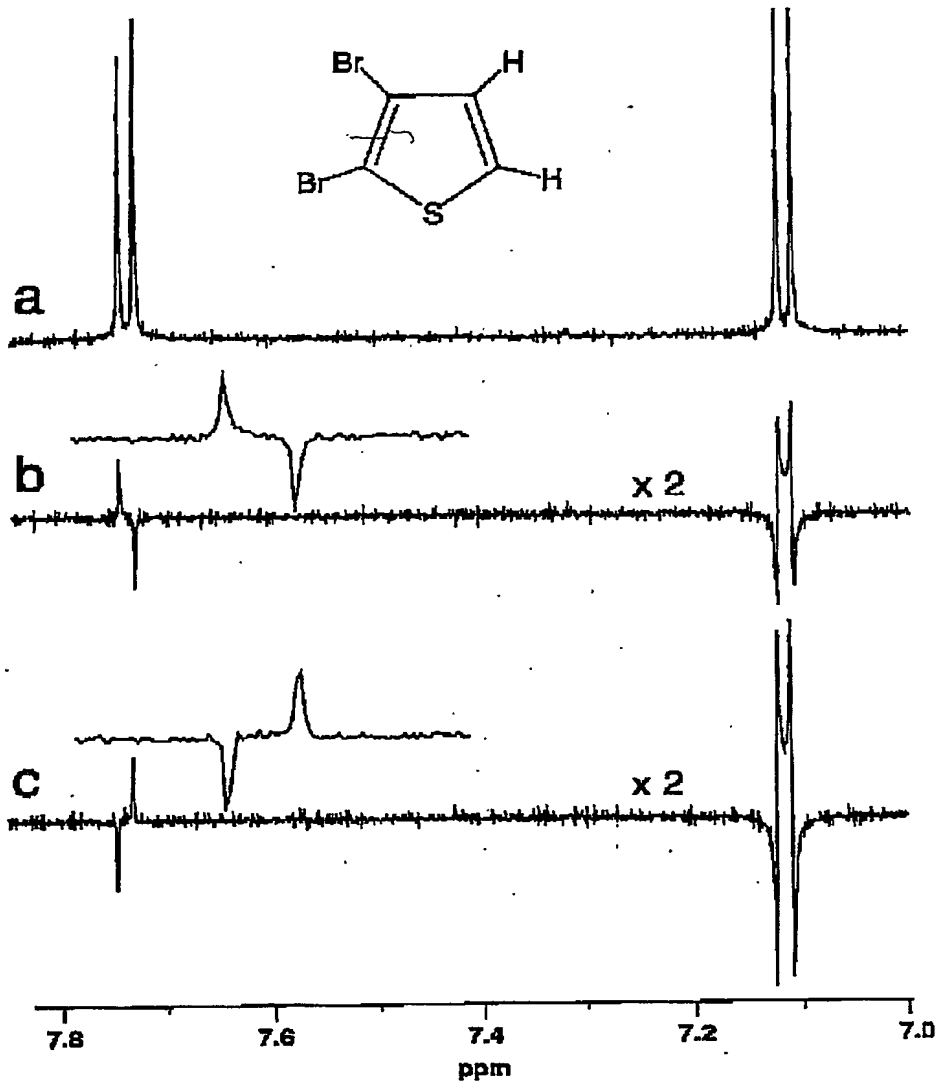


Fig.2

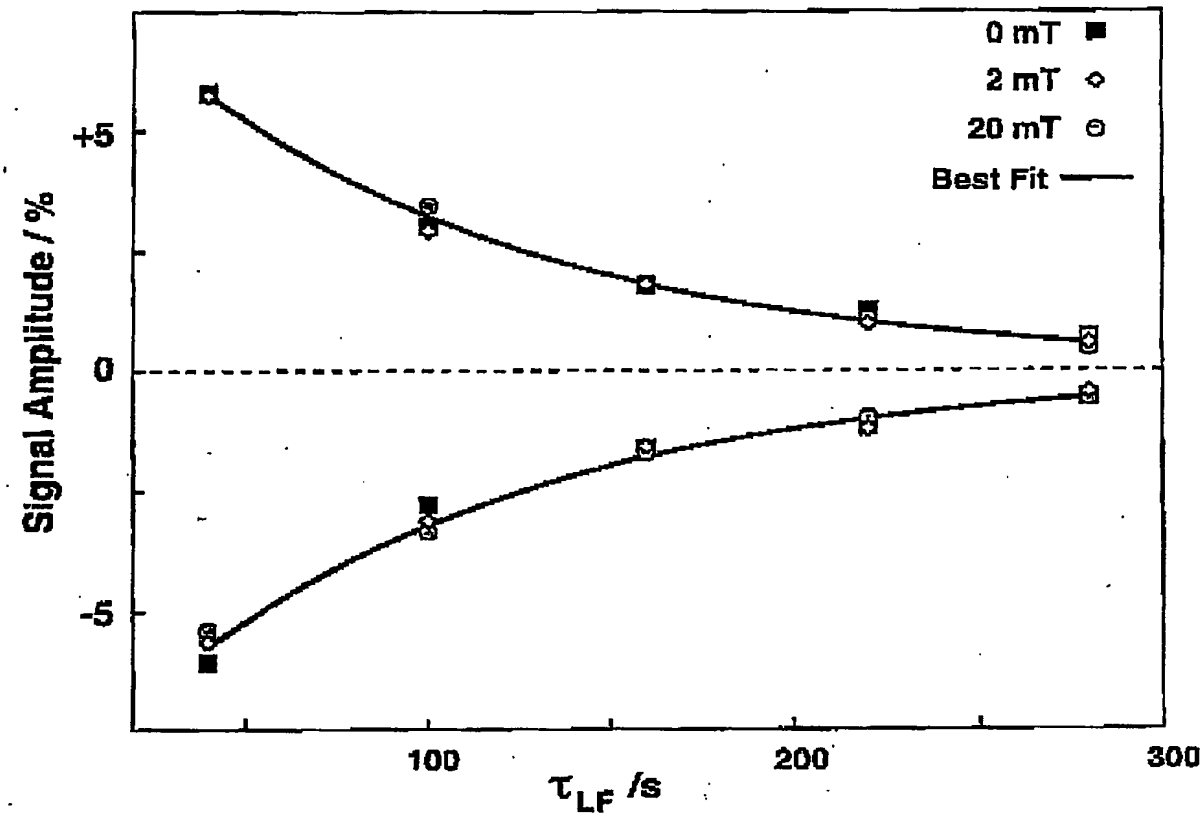
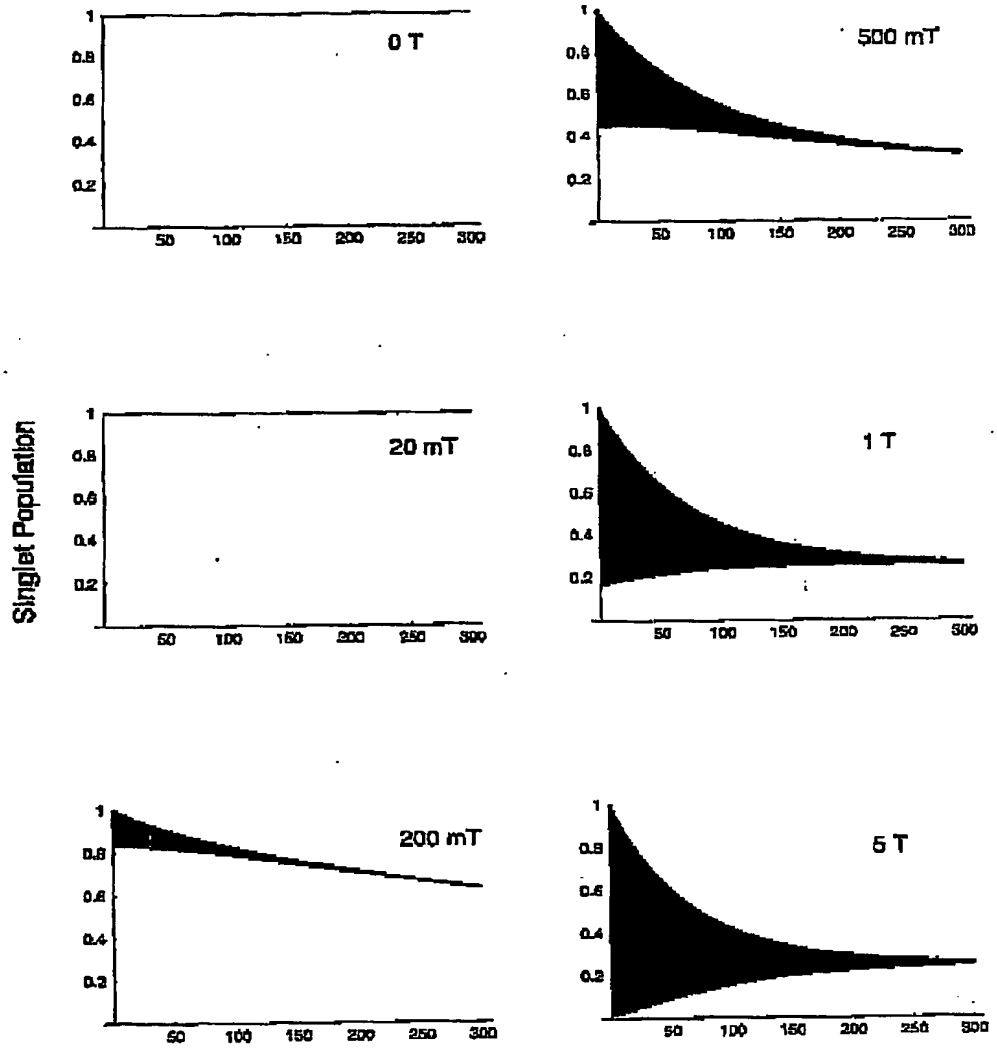


Fig.3



. . Supporting Material Fig.1

Supporting Material

Radiofrequency Pulse Sequences

Sequence A_+

The pulse sequence A_+ is given by the two-pulse sequence $90_0 - \tau_1 - 90_{-90}$, where the symbol β_ϕ denotes a strong, non-selective pulse with flip angle β and phase ϕ (both angles are specified in degrees, and the phases taken into account the sign of the precession and the radio-frequency mixing scheme (1,2)). The delay τ_1 is set to the value $\tau_1 = |\pi/\omega_\Delta^{\text{high}}|$, where

$$\omega_\Delta^{\text{high}} = -\gamma B_{\text{high}}(\delta_2 - \delta_1) \qquad [1]$$

For the experimental results, the delay was set to the value $\tau_1 = 2.05\,\text{ms}$.

Standard spin operator theory (3,4) shows that in the case $\delta_2 > \delta_1$ and $\gamma > 0$, the pulse sequence A_+ has the approximate propagator

$$U(A_+) = \exp\{-i\frac{\pi}{2}(I_{1x} + I_{2x})\}\exp\{+i\pi I_{2y}\} \qquad [2]$$

neglecting the effect of the J-coupling during the interval τ_1. This assumption is valid in the case that the chemical shift frequency difference is much larger than the J-coupling (weak coupling approximation). The transformations of

the individual state kets are

$$\begin{aligned} U(A_+)|\alpha\alpha> &= -|\alpha\beta> \\ U(A_+)|\beta\alpha> &= -i|\alpha\alpha> \\ U(A_+)|\alpha\beta> &= -i|\beta\beta> \\ U(A_+)|\beta\beta> &= |\beta\alpha> \end{aligned} \qquad [3]$$

which leads to the exchanges of populations sketched in Fig.1c. The population of the $|\alpha\beta>$ state at time point ②is derived from the thermal equilibrium population of the $|\alpha\alpha>$ state.

Sequence A_-

The pulse sequence A_- is given by the two-pulse sequence $90_{180} - \tau_1 - 90_{-90}$. The sequence differs from A_+ by a 180° phase shift of the first pulse. The spin propagator for this sequence, under the weak-coupling approximation, is

$$U(A_-) = \exp\{+i\frac{\pi}{2}(I_{1z} + I_{2z})\} \exp\{+i\pi I_{1y}\} \qquad [4]$$

which leads to the following ket transformations:

$$\begin{aligned} U(A_-)|\alpha\alpha> &= -|\alpha\beta> \\ U(A_-)|\beta\alpha> &= i|\beta\beta> \\ U(A_-)|\alpha\beta> &= i|\alpha\alpha> \\ U(A_-)|\beta\beta> &= |\beta\alpha> \end{aligned} \qquad [5]$$

The population of the $|\alpha\beta>$ state at time point ②is derived from the thermal equilibrium population of the $|\beta\beta>$ state.

Since the $|\alpha\beta>$ state is connected adiabatically to the low-field singlet, the sequences A_+ and A_- may be used to prepare low-field singlet states at time point ③ with populations deviating from the mean in opposite senses.

Sequence B

The pulse sequence B is given by $90_0 - \tau_2 - 180_{90} - \tau_3 - 90_{45}$, with pulse sequence delays given by $\tau_2 = |\pi/(2\omega_\Delta^{\mathrm{high}})| + |1/(4J)|$ and $\tau_3 = |1/(4J)|$. For the experimental results, the delays were set to the values $\tau_2 = 44.64$ ms and $\tau_3 = 43.61$ ms. This sequence is designed to separate cleanly the interesting NMR signals deriving from the low-field singlet state from the uninteresting signals generated by relaxation of the spin system during the second transport interval.

From standard spin-operator theory (3,4), the propagator $U(B)$ for this pulse sequence transforms the population operator of state $|\alpha\beta>$ into a superposition of many operator terms, including some that represent antiphase single-quantum coherences:

$$\begin{aligned} U(B)I_1^\alpha I_2^\beta U(B)^\dagger \;=\; & -\frac{1}{4}\exp\{-i\pi/4\}I_1^- I_2^\alpha - \frac{1}{4}\exp\{+i\pi/4\}I_1^\alpha I_2^- \\ & +\frac{1}{4}\exp\{-i\pi/4\}I_1^- I_2^\beta + \frac{1}{4}\exp\{+i\pi/4\}I_1^\beta I_2^- + \ldots \end{aligned} \qquad [6]$$

The (−1)-quantum coherences represented by operators $I_1^{\alpha}I_2^{-}$ and $I_1^{\beta}I_2^{-}$ generate the diagnostic antiphase signals near the chemical shift δ_2.

Sequence B gives rise to no net signals at the chemical shift δ_2 when applied to in-phase Zeeman magnetization, created by T_1 relaxation during the second transport interval $\tau_{transp}^{(2)}$. This may be seen from the transformations

$$\begin{aligned} U(B)I_{1z}U(B)^{\dagger} \;=\; & -\frac{1}{2}\exp\{+i\pi/4\}I_1^{+}I_2^{\alpha} - \frac{1}{2}\exp\{-i\pi/4\}I_1^{-}I_2^{\alpha} \\ & +\frac{1}{2}\exp\{+i\pi/4\}I_1^{+}I_2^{\beta} + \frac{1}{2}\exp\{-i\pi/4\}I_1^{-}I_2^{\beta} \end{aligned} \qquad [7]$$

and

$$U(B)I_{2z}U(B)^{\dagger} = \frac{1}{2}I_1^{+}I_2^{+} + i\frac{1}{2}I_1^{+}I_2^{-} - i\frac{1}{2}I_1^{-}I_2^{+} + \frac{1}{2}I_1^{-}I_2^{-} \qquad [8]$$

Partially-relaxed Zeeman magnetization of spin I_1 only generates signals at the chemical shift δ_1, while partially-relaxed Zeeman magnetization of spin I_2 only generates multiple-quantum coherences, which do not generate a signal at all. In principle, cross-correlated relaxation (5) during the transport interval could also generate spurious signals at the shift δ_2. Such signals would not change sign when exchanging the sequences A_+ and A_-, and appear to be negligible in the system studied here.

Sample

The sample was 500 microlitres of a 17.7 mM solution of 2,3-dibromothiophene in DMSO-d^6, contained in a 5 mm high-resolution NMR tube equipped with a Young valve to facilitate degassing. The sample contained a water impurity in approximately 27 mM concentration. The sample was subjected to three freeze-pump-thaw degassing cycles, each lasting over 30 minutes, to remove dissolved oxygen.

Experimental Procedure

The NMR experiments were performed on a Varian Infinity+ 400 MHz NMR system at a field of $B_{\rm high} \cong 9.4\,{\rm T}$, using an actively-shielded 89 mm bore magnet. A standard 5 mm high-resolution NMR probe was used. The 90° pulse duration was around 5 μs.

The sample was transported between the high and low fields using the standard pneumatic sample elevator. The air pressure of the elevator was increased during the lift operation, and reduced during the insert operation, in order to accelerate the transport times. With practice we could achieve fairly reproducible transport times of $\tau^{(1)}_{\rm transp} = 9 \pm 1\,{\rm s}$ and $\tau^{(2)}_{\rm transp} = 13 \pm 2\,{\rm s}$, measured from when the sample is fully loaded into the coil to when the sample is outside the magnet bore.

The field B_{low} were estimated using a Hall-effect Gauss meter, with zero field calibrated to an accuracy of approximately $10\,\mu$T using a mu-metal shield far from the magnet.

For a storage field $B_{low} = 20\,$mT, the sample was elevated to the top of the magnet bore and allowed to remain there for the time τ_{LF}. For the lower fields, the sample was carried physically to calibrated locations within the stray field of the magnet, or in the case $B_{low} = 0$, into the magnetically-shielded chamber.

Numerical Simulations

The Liouville-von Neumann equation may be integrated using the low field Liouvillian $\hat{\bar{L}}_{LF}$ (Eq.[8] of the main manuscript) to predict the trajectory of the singlet population as a function of magnetic field and molecular motional parameters.

For 2,3-dibromothiophene, the distance between the proton nuclei was estimated to be $r = 253$ pm. The spin-lattice relaxation in high field was assumed to be dominated by intramolecular dipole-dipole relaxation, modelled as the isotropic rotational diffusion of a rigid molecule. With this model, the observed T_1 value of 20 s corresponds to a rotational correlation time of $\tau_c = 15\,$ps.

Dynamic simulations of the singlet state population as a function of time and field are shown in Fig.1. At low values of the field B_{low}, the singlet state Fig.1

population is predicted to be time-independent over a timescale of hundreds of seconds. As the storage field is increased beyond around 200 mT, a rapid low-amplitude oscillation of the singlet state population is superimposed on a slower decay. At higher fields the amplitude of the oscillations becomes very large and at long times the population equilibrates among the four available states, indicating the loss of all spin order.

The experimental results show a significant damping of singlet spin order on the timescale of 100 seconds for fields much less than 200 mT. This indicates the participation of relaxation mechanisms other than intramolecular dipole-dipole coupling. Intermolecular dipole-dipole relaxation involving water protons or protons on different DBT molecules is likely to be the major singlet damping mechanism.

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Figure Captions.

Fig.1. Simulated trajectories of the singlet state population for the case $\delta_1 = 7.11\,\text{ppm}$, $\delta_2 = 7.72\,\text{ppm}$, $J = 5.7\,\text{Hz}$, $r = 253$ pm and $\tau_c = 15\,\text{ps}$. The values of B_{low} are shown in the figure. The population of the singlet state is tracked starting from an initial condition in which the singlet is 100% populated and all the other states have zero population. At high storage fields, the singlet state population is predicted to oscillate rapidly and decay towards a final value of 25%, representing an equal distribution of populations amongst the four states. The dark areas indicate very rapid oscillations of the singlet population.